The image offsets, x_0 and y_0, can be measured from the shift in the two-dimensional correlogram, since:

$$C_{1,2} = f_1(x,y) \otimes f_2(x,y) = f_1(x,y) \otimes f_1(x,y) \otimes \delta(x - x_0, y - y_0).$$

The Fourier Transform provides a convenient method for accomplishing the convolution of two images, as seen in the theorem:

$$f_1(x,y) \otimes f_2(x,y) = F^{-1}\left[F_1(\omega_x,\omega_y) \cdot F_2(\omega_x,\omega_y)\right]$$

$$where:$$

$$F(\omega_x,\omega_y) = \text{Fourier Transform of } f(x,y),$$

$$F^{-1}[X(\omega_x,\omega_y)] = \text{Inverse Fourier Transform of } X(\omega_x,\omega_y).$$

In the frequency domain, then, the two-dimensional convolution becomes a matrix multiplication of the spectra of the two images. The two-dimensional correlogram is obtained by performing the inverse Fourier Transform of the product.

Because objects may occupy only a few pixels, and because the resolution of the imaging system may be on the order of a pixel width, alignment of one image to the next to a resolution of a fraction of a pixel width is necessary for accurate crosstalk correction. The true peak of the correlogram will rarely be centered on a pixel. The true peak can be located, however, by analyzing the shape of the region around the pixel of maximum amplitude. If an accurate equation can be defined for the correlogram amplitude in that region, the true location of the peak can be found to a resolution better than a single pixel width.

For cross talk correction an image is reconstructed for alignment by application of two-dimensional interpolation. In this process, once the image has been aligned with the reference image to the nearest pixel, the new amplitude value for each pixel is computed as the weighted sum of a group of surrounding pixels. The values of the weighting coefficients for the interpolation depend on the amount of shifting to be accomplished in the vertical direction and the horizontal direction. These shift distances will be less than one pixel width.

With aligned data images in place, the crosstalk correction can be accomplished. FIGURE 27 illustrates imagery before and after cross talk correction. Imagery 1601 clearly shows objects imaged upon a detector in the presence of uncorrected cross talk. Imagery 1602 illustrates the same imagery after cross talk has been removed. Depending upon the means for used for tagging reporters, fluorochromes, quantum dots etc., it may or may not be necessary to remove cross talk from imagery projected upon the detector(s) before decoding.

Analyzing Reporter labeled Beads Using the Flow Imaging System of the Present Invention

Decoding of reporter labeled beads by the second embodiment of the flow imaging system is depicted in FIGURE 17. Each bead and its associated reporters are simultaneously imaged multiple times across several rows covering a horizontal region on the detector, each image corresponding to a different emission, reflectance or absorbance band. In FIGURE 17, the solid substrate, or carrier bead 346, is indicated by the large circle, while reporters 348a, 348b, 348c and 348d are depicted as a smaller circle bound to the larger bead.

Each of these reporters can be seen in the (Optional) reference channel of the detector. The imagery in this section can be generated by light source scatter signals from the beads and reporters or by absorbance signals generated from band limited brightfield illumination. For reporters using fluorescence to uniquely identify their optical signature, the reference channel is optional if each reporter contains at least one type of fluorochrome. A binding signal is indicated by a filled circle 346b in the binding signal section of the detector. A signal in this channel indicates that an analyte has bound to the bead as a result of the assay. Further, the intensity of the signal in the binding signal channel can be used to further characterize the binding event to determine the quantity of the analyte present in the assay.

Reporter signals are imaged in the remaining blue, green, yellow and red channels of the detector. The present invention maintains the relative positions of all reporter imagery in each channel. Thus reporter 348a is associated with imagery 342a in the yellow channel and imagery 342b in the red channel of the detector. Reporter 348a contains both yellow and red fluorochromes, and since no other imagery appears in the blue or green channels, no other fluorchromes are present in reporter 348a. Similarly, reporter 348b is associated with imagery 343a in the blue channel and imagery 343b in the red channel, reporter 348c is associated with imagery 344a in the green channel and imagery 344b in the yellow channel, and reporter 348d is associated with imagery 345a in the blue channel and imagery 345b in the green channel.

Such reporter imagery must be matched to a known reporter legend, to identify an object that is associated with a specific reporter. It should be noted that reporter labeled beads can be associated with many different types of objects, such as cells, chemical or biological compounds. As discussed in the Background of the Invention, certain types of DNA related research involves the preparation of libraries of synthesized compounds, such as an oligo library generated using the SAP technique described above. Such compounds are produced by combining a plurality of subunits

(for example, nucleotide bases) together to form a larger compound (such as an oligomer). While several different methods can be employed to produce such compounds, if each subunit is individually tagged with an identifying characteristic, such as a fluorchrome, then the presence of a reporter encodes the identity of the subunit in the compound, and its location relative to other subunits, based on a known reporter legend. FIGURE 19 illustrates a reporter legend, that was generated when a oligo library comprised of nucleotide bases was generated. According to the reporter legend of FIGURE 19, the presence of the yellow-red reporter 348a indicates cytosine (C) in a first position in the oligo bound to bead 346. The presence of the blue-green reporter 348d indicates an adenine (A) in the last position of the oligo bound to bead 346. In this manner, all positions of an oligo can be determined by identifying the color combinations present in each reporter. Using the present invention it is very straight forward to decode the "CGTA" oligo sequence synthesized on bead 346. Furthermore, the presence of the binding signal (see FIGURE 17) indicates whether or not the complimentary "GCAT" sequence has bound to the bead during the assay. It should be noted that while reporter labeled beads will be useful in identifying all the subunits of a compound that include individually labeled subunits, it is anticipated that it will also be useful to label (and read) compounds not at the subunit level, but just at the compound level. Thus the present apparatus and method is not limited to imaging and reading only compounds that include individually labeled subunits.

The decoding process described above is identical for the fourth and fifth embodiment of the flow imaging system described above, but each spectral band is imaged onto a separate detector. Although this embodiment requires more detectors, each detector can have relatively few pixel columns since there is only one spectral band per detector. This embodiment is also more optically efficient because the light reaching the detector passes the filter set once, unlike the previous embodiment where light must pass through each filter twice before exiting the dispersing component and reaching the detector.

The decoding process used for the first embodiment is similar in concept to the process employed for the second through fourth embodiments, except that the reporter images must be generated by first deconvolving emission spectra of the fluorochromes used from the imagery on the detector. Deconvolution of the emission spectra from the bead image requires significant image processing, which is a disadvantage for real-time analysis. However, the advantage of this first embodiment is that it potentially provides the highest spectral discrimination power, but uses only one detector and one optical dispersing element, reducing system cost.